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Genetic relatedness in two Southern sea lion (*Otaria flavescens*) rookeries in the southwestern Atlantic

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The southern sea lion, *Otaria flavescens* (Shaw, 1800), is distributed along the coast of South America, from Torres (29°20′ S 49°43′ W) in southern Brazil in the Atlantic Ocean (Rosas et al. 1994) to Cape Horn in the extreme south, and from Cape Horn to Zorritos (4° S) in northern Perú in the Pacific Ocean (Riedman 1990). The northernmost breeding grounds are along the coasts of Uruguay (Isla de Lobos, Cabo Polonio, and La Coronilla). A total of 15,000 individuals was estimated for this area. To the north of the Uruguayan breeding grounds, in southern Brazil, there are only two non-breeding rookeries where subadult males predominate. Seasonal movements have been documented for Rio Grande do Sul coast (Rosas et al. 1994). In addition, erratic records for the species have been reported from Rio de Janeiro (23° S) and even to 13° S (Castello 1984) but always by solitary individuals.

In Argentina, they breed along the Patagonian coast, from Punta Bermeja at 41°08′ S to 55° S on Tierra del Fuego Island. Presently, there are 54 breeding and non-breeding rookeries with a total of 51,000 individuals up to 47°05′ S, 66°16′ W (Reyes et al. 1999). Individual movements showing seasonal patterns have been demonstrated (Crespo 1988; Crespo and Pedraza 1991). To the north of the Patagonian grounds, there are only two subadult male rookeries in Buenos Aires Province at the Mar del Plata (38° S) and Quequén harbours 38°30′ S), as well as a breeding rookery at Isla Trinidad (39° S).

Biochemical-genetic data have been used as a powerful tool for studying population biology; moreover, they can provide much information about the population structure of a species. Taking into account the reduced information available with respect to migrations and interchange of individuals, the aim of this study was to analyse the genetic variability in the southern sea lion in two rookeries: Isla de Lobos, Uruguay and Punta Norte, Península Valdés.

Collection of samples: Blood samples were collected from 70 southern sea lion pups (less than 30 days old), during the 1992/93 breeding season, from two rookeries 1,300 km distant from each other: Isla de Lobos, Maldonado, Uruguay (35 blood samples) (35°02′ S, 52°55′ W) and Punta Norte, Península Valdés, Chubut, Argentina (35 blood samples) (42°04′ S, 63°47′ W) (Fig. 1). All pups sampled were selected at random from breeding harems at both localities. About 2 to 5 ml of heparinized peripheral blood was collected

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Fig. 1. Location of the study areas and of the geographical names mentioned in the text. ● Reference rookeries, ▲ Sites of sample collection.

from the extradural vein from the lumbar region. After blood extraction, pups were returned to their mothers.

Genetic analysis: Blood samples were processed in situ, in the field. Plasma and erythrocytes stored in liquid nitrogen were subjected to horizontal 5 %–7 % polyacrylamide gel electrophoresis (PAGE). Only 64 samples from the 70 collected were included for the electrophoretic interpretation. Nine protein systems representing 10 putative loci were analysed: lactate dehydrogenase A and B (LDHA, LDHB), malate dehydrogenase (MDH), phosphogluconate dehydrogenase (PGD), isocitrate dehydrogenase (IDH), superoxide dismutase (SOD), glutamate oxalacetate transaminase (GOT), and esterase D (ESD) from erythrocytes, and transferrin (TF) and albumin (ALB) from plasma. The proteins studied, the electrophoretic methods and staining procedures are given in table 1. To locate TF patterns, electrophoresis gels were run with human standards (HSA) and the genotypes were interpreted by direct comparison with them.

Genotypic frequencies were tested by maximum likelihood for Hardy-Weinberg equilibrium. Differences in gene frequencies between the two rookeries were tested by means of a two-tailed binomial test (Zar 1996). Observed and expected mean gene diversity values at population level (H_o and H_e) and Nei (1978) genetic distance (D) were calculated by using the Genetic Data Analysis (GDA) computer program (Lewis and Zaykin 1997).

From the 10 examined loci, nine were monomorphic and six of them as shown in Gales et al. (1989): GOT, LDHA, LDHB, MDH, PGD, SOD. Only TF was polymorphic for both sea lion rookeries, and curiously, it was monomorphic in southern elephant seals (Gales et al. 1989).

Table 1. Screened proteins in *Otaria flavescens*. Optimal buffer systems: 1) Tris-maleic-EDTA-magnesium chloride, pH 7,4 (SHAW and PRASAD 1970); 2) Tris-EDTA-citric acid, pH 7.0 (SHAW and PRASAD 1970); 3) Phosphate-EDTA-citrate, pH 6.9 (SCHNEIDER 1988); 4) Phosphate-citric acid, pH 5.9 (HARRIS and HOPKINSON 1976); 5) Lithium hydroxide-boric acid, pH 8.3 (BOUMAN and BEARN 1965). Staining methods references: a) HARRIS and HOPKINSON (1976); b) HILLIS and MORITZ (1990); c) SCHNEIDER (1988).

System	Abbreviature and enzyme commission number	Optimal buffer system	Staining method reference
1. Erythrocyte enzymes			
Lactate dehydrogenase	LDH 1.1.1.27	1	a
Malate dehydrogenase	MDH 1.1.1.37	2	a
Phosphogluconate dehydrogenase	PGD 1.1.1.44	3	a
Isocitrate dehydrogenase	IDH 1.1.1.42	4	a
Superoxide dismutase	SOD 1.15.1.1	4	a
Glutamate oxalacetate transaminase	GOT 2.6.1.1	2	b
Esterase D	ESD 3.1.1.1	3	a
2. Serum proteins			
Transferrin	TF	5	c
Albumin	ALB	5	c

The same alleles of TF were found at PN and IL. Two alleles and three different electrophoretic TF phenotypes were observed. Both TF 1 and TF 2 bands showed slower electrophoretic mobility than that of human TF C. The heterozygotes TF 1–2 showed a two-banded pattern, consistent with the monomeric molecular structure of this protein.

Under the assumption that genotypes are determined by two different alleles, the genotype distribution in both populations was in Hardy-Weinberg equilibrium according to the maximum likelihood estimation (Tab. 2). The allele Tf^2 was the most frequent in both sample sites. The two tailed binomial test showed that the gene frequencies were significantly different for the stocks ($\varepsilon = 2.5897$, $p < 0.01$). The sample size could explain in part the observed differences in the gene frequencies. TF is usually a good molecular marker that is polymorphic in many mammals (GOODMAN et al. 1965; SHAUGHNESSY 1969; HERZOG et al. 1991; HARTL and FERRAND 1993), and as it showed polymorphism in the present study it could be used as a molecular marker according to the different frequencies found.

Observed genetic heterozygosity for southern sea lions at Isla de Lobos was $H_o = 0.027$ and $H_o = 0.003$ for Punta Norte. These H values are in the extremes of previous values given for other pinniped species (GALES et al. 1989). It has been stated that

Table 2. Observed transferrin phenotypes, allele frequencies, expected and observed mean heterozygosity (He and Ho) and an estimate of the fixation index (f) in the two studied rookeries of *Otaria flavescens*.

Rookery	Number of individuals of each phenotype			Gene frequencies		He	Ho	f
	1-1	1-2	2-2	Tf^1	Tf^2			
PUNTA NORTE	0	1	33	0.0147	0.9853	0.002941	0.002941	0.00000
ISLA DE LOBOS	2	9	19	0.2167	0.7833	0.032542	0.026667	0.18309

marine mammals as a group are low in genetic variation (Saughnessy 1975; McDermid and Bonner 1975; Lidicker et al. 1981; Gales et al. 1989); however the H value for PN is extremely low, probably as a consequence of a bottleneck effect. Between the 30's and the 50's, the southern sea lion population in northern Patagonia was reduced to about 10 % of its original size remaining in a stable condition between 1975 and 1991. They were heavily exploited, mainly from Península Valdés and Tierra del Fuego, by national permissioners for leather and oil. In Península Valdés, the population was reduced during this period from 200,000 to less than 15,000 individuals (Crespo and Pedraza 1991). Presently, there are 35,000 individuals and the population is increasing at a rate of 3.5 % per year (Crespo pers. comm.).

On the other hand, the H value of IL is one of the highest found in pinnipeds according to Gales et al. (1989). One reason may be that another pinniped species, the southern fur seal *Arctocephalus australis*, has been the main exploited species in Uruguay instead of *O. flavescens*. However, Vaz-Ferreira (1979) stated 30,000 individuals of *O. flavescens* for 1954, and between 1986 and 1990 Lima and Batallés (pers. comm.) estimated 15,000 individuals, reaching the lowest number ever known for this stock and with a decreasing trend.

The Nei genetic distance obtained between Punta Norte and Isla de Lobos was D = 0.003. This is a low value compared with intraspecific values found from populations of other mammalian species (Selander and Johnson 1973). There are no previous reports of genetic distances for conspecific pinnipeds except for *M. leonina* from Macquarie and Heard Islands (D = 0.007, Gales et al. 1989).

Taking into account that individual movements between Patagonian and Uruguayan stocks have been recorded from marked animals (Lorenzani and Lorenzani, pers. comm.), this suggests a high mobility of individuals between rookeries, especially considering the low number of marked animals from sites with several hundreds or thousands individuals. In this opportunity, the presence of the same fixed allele at each of the nine monomorphic loci and the low value of genetic distance leads to the conclusion that both rookeries belong to the same population in which gene flow is currently occurring.

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